

IN THE CLAIMS:

1. **(Currently Amended)** An isolated nucleic acid fragment selected from the group consisting of:
 - (a) an isolated nucleic acid fragment encoding a polypeptide with nitrilase activity as set forth as SEQ ID NO:5;
 - (b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under highly stringent hybridization conditions of 6X SSC (1M NaCl), ~~50~~40 to 45 % formamide, 1 % SDS at 37 °C, and a wash in 0.1X ~~5X~~ to 1X SSC at ~~60 to 65~~55 to 60 °C; and
 - (c) an isolated nucleic acid fragment that is completely complementary to (a) or (b).
2. **(Currently Amended)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 90~~80~~% identity as ~~determined by the Needleman and Wunsch algorithm under default parameters~~ compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
3. **(Withdrawn)** An isolated nucleic acid fragment encoding a nitrilase enzyme, selected from the group consisting of:
 - (a) an isolated nucleic acid fragment selected from the group consisting of SEQ ID NO: 4, and SEQ ID NO:16;
 - (b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under stringent hybridization conditions of 6X SSC (1M NaCl), 40 to 45% formamide, 1 % SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60 °V' smf
 - (c) an isolated nucleic acid fragment that is completely complementary to (a) or (b).
4. **(Withdrawn)** An isolated nucleic acid sequence encoding a nitrilase enzyme selected from the group consisting of SEQ ID NO:4, and SEQ ID NO:15.
5. **(Cancelled)**
6. **(Withdrawn)** A polypeptide encoded by the nucleic acid fragments of any of Claims 1, 2, 3, or 4.
7. **(Withdrawn)** A polypeptide according to Claim 6 having the amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:14.
8. **(Withdrawn)** The polypeptide of Claim 6 further characterized by nitrilase activity on nitrile-containing substrates selected from the group consisting of aliphatic nitriles and aromatic nitriles.

9. **(Previously presented)** A chimeric gene comprising the isolated nucleic acid fragment of Claims 1 or 2 operably linked to suitable regulatory sequences.
10. **(Previously presented)** A plasmid pnitex2 contained in *E. coli* SS1001 having the designation ATCC PTA-1177.
11. **(Original)** An expression cassette comprising the chimeric gene of Claim 9.
12. **(Previously presented)** The expression cassette of Claim 11 comprising plasmid pnitex2.
13. **(Original)** A transformed microorganism comprising the chimeric gene Claim 9.
14. **(Original)** A transformed microorganism comprising the plasmid of Claim 10.
15. **(Original)** A transformed microorganism comprising the expression cassette of Claim 11.
16. **(Original)** The transformed microorganism of Claim 15 wherein the expression cassette is chromosomally integrated.
17. **(Original)** The transformed microorganism of Claim 16 further comprising suitable regulatory sequences.
18. **(Original)** The transformed microorganism of Claim 17 wherein the suitable regulatory sequences comprise
 - a) at least one promoter selected from the group consisting of the tryptophan operon promoter P_{trp} of *E. coli*, a lactose operon promoter Plac of *E. coli*, a P_{tac} promoter of *E. coli*, a phage lambda right promoter P_R, a phage lambda left promoter P_L, a T7 promoter, a promoter of the AOX1 gene from *Pichia pastoris*, and a promoter of the GAP gene from *Pichia pastoris*, or is at least one strong promoter selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Pichia*, *Zygosaccharomyces*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*, and
 - b) at least one ribosome binding site from a phage lambda CII gene or selected from the group consisting of ribosome binding sites from a gene of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*.
19. **(Original)** The transformed microorganism of Claim 18, wherein the host microorganism is selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*,

Klebsiella, Salmonella, Lactobacillus, Aspergillus, Saccharomyces, Zygosaccharomyces, Pichia, Kluyveromyces, Candida, Hansenula, Dunaliella, Debaryomyces, Mucor, Torulopsis, Methylobacteria, Bacillus, Escherichia, Pseudomonas, Rhizobium, and Streptomyces.

20. **(Previously presented)** A transformed microorganism *E. coli* SS1001 having the designation ATCC PTA-1177.
21. **(Cancelled)**
22. **(Cancelled)**
23. **(Cancelled)**
24. **(Withdrawn)** A method to enzymatically convert nitrile-containing substrates to a carboxylic acid, the method comprising:
 - (a) contacting, under suitable conditions, a transformed heterologous host expressing the polypeptide of Claim 4 with a nitrile-containing substrate; and
 - (b) optionally collecting the carboxylic acid produced in step (a).
25. **(Withdrawn)** The method of Claim 24, wherein the nitrile-containing substrate is a dinitrile of the formula NC-R-CN where R is an alkylene group having from 1 to 10 carbon atoms.
26. **(Withdrawn)** The method of Claim 25, wherein the nitrile-containing substrate is 2-methylglutaronitrile.
27. **(Withdrawn)** A method to enzymatically convert nitrile-containing substrate(s) to carboxylic acid(s), the method comprising:
 - (a) contacting, under suitable conditions, a transformed heterologous host comprising the chimeric gene of Claim 9 with nitrile-containing substrate(s); and
 - (b) optionally collecting the carboxylic acid produced in step (a).
28. **(Withdrawn)** The method of Claim 27, wherein the nitrile-containing substrate is a dinitrile of the formula NC-R-CN where R is an alkylene group having from 1 to 10 carbon atoms.
29. **(Withdrawn)** The method of Claim 27, wherein the nitrile-containing substrate is 2-methylglutaronitrile.
30. **(Withdrawn)** The method of Claim 27 wherein the suitable regulatory sequences of the chimeric gene comprise an inducible promoter.
31. **(Withdrawn)** The method of Claim 30, the suitable conditions of step (a) further comprising the presence of an inducer of the inducible promoter.
32. **(Withdrawn)** The method of enzymatically converting 2-methylglutaronitrile to the corresponding carboxylic acid, the method comprising:
 - (a) contacting, under suitable conditions, *E. coli* SW91 designated ATCC PTA-1175 with 2-methylglutaronitrile; and

(b) optionally collecting the carboxylic acid produced in step (a).

33. **(Withdrawn)** An improvement to the process for preparing five-membered ring lactams or six-membered ring lactams from aliphatic α,ω -dinitriles comprising:

- (a) contacting an aliphatic α,ω -dinitrile in an aqueous reaction mixture with an enzyme catalyst, whereby the aliphatic α,ω -dinitrile is converted to an ω -cyanocarboxylic acid ammonium salt;
- (b) contacting the aqueous product mixture resulting from step (a) with hydrogen and a hydrogenation catalyst, whereby the ω -cyanocarboxylic acid ammonium salt is converted directly to the corresponding lactam without isolation of the intermediate ω -cyanocarboxylic acid, ω -cyanocarboxylic acid ammonium salt, ω -aminocarboxylic acid, or ω -aminocarboxylic acid ammonium salt; and
- (c) recovering the lactam from the aqueous product mixture resulting from step (b),

the improvement comprising in step (a) contacting an aliphatic α,ω -dinitrile in an aqueous reaction mixture with an enzyme catalyst selected from the group consisting of

- (1) *E. coli* SW91 having the designation ATCC PTA-1175;
- (2) *E. coli* DH5a: pnit4 having the designation ATCC PTA-1176;
- (3) *E. coli* SS1001 having the designation ATCC PTA-1177; and
- (4) *E. coli* SS1002 containing plasmid pnitex2, and
- (5) *E. coli* SS1011 containing plasmid pnitex2.

34. **(Withdrawn)** The process of Claim 33 wherein the aliphatic α,ω -dinitrile has the formula



where $a = 0$ or 1 , where $X = \text{hydrogen}$ when $a = 1$, and $R = \text{H}$, alkyl or substituted alkyl, or alkenyl or substituted alkenyl, or alkylidene or substituted alkylidene, and where $n = 1$ or 2 .

35. **(Withdrawn)** The method of Claim 34 wherein the α,ω -dinitrile is 2-methylglutaronitrile.

36. **(Withdrawn)** The process of Claim 33 wherein the aliphatic α,ω -cyano is unsymmetrically substituted at the α -carbon atom, and the enzyme catalyst is characterized by aliphatic nitrilase activity that produces the ω -cyanocarboxylic acid ammonium salt resulting from regioselective hydrolysis of the ω -cyano group, thereby producing only one of the two possible lactam products during step (b).

37. **(Withdrawn)** The process of Claim 33 further comprising adding, before step (b), ammonium hydroxide, ammonia gas, or methylamine to the aqueous product mixture containing the ω -cyanocarboxylic acid ammonium salt

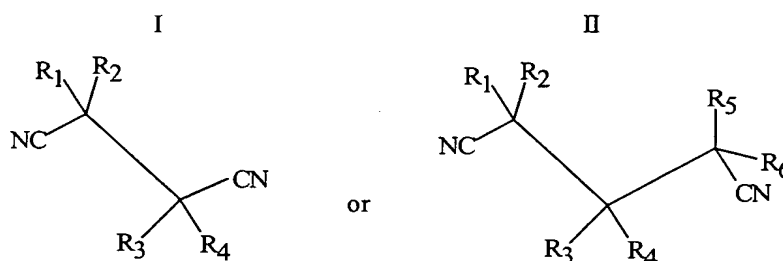
38. **(Withdrawn)** The process of Claim 37 wherein the amount of ammonium hydroxide, ammonia gas, or methylamine added to the aqueous product mixture is from 0 to 4 molar equivalents relative to the amount of ω -cyanocarboxylic acid ammonium salt present.

39. **(Withdrawn)** The process of Claim 38 wherein methylamine is added to the aqueous product mixture containing the ω -cyanocarboxylic acid ammonium salt before step (b) and the product of step (b) is an N-methylactam.

40. **(Withdrawn)** The process of Claim 33 wherein in step (b) the temperature of the aqueous product mixture is from 45 °C to 200 °C.

41. **(Withdrawn)** The process of Claim 33 for preparing five-membered ring lactams or six-membered ring lactams from aliphatic α,ω -dinitriles, wherein:

the aliphatic α,ω -dinitrile is of either formula:



where R₁ and R₂ are both H, and

R₃, R₄, R₅, and R₆ are each independently selected from the group consisting of H, alkyl or substituted alkyl, or alkenyl or substituted alkenyl, or R₃ and R₄ taken together are alkylidene or substituted alkylidene, or independently R₅ and R₆ taken together are alkylidene or substituted alkylidene.

42. **(Withdrawn)** The method of Claim 33 or 41 wherein the enzyme catalyst is in the form of whole microbial cells immobilized in or on an insoluble support.

43. **(Withdrawn)** A method for using a native microbial gene encoding a protein characterized by a nitrilase activity on nitrilase-containing substrates to obtain a mutated microbial gene encoding a protein characterized by an increased specific nitrilase activity on nitrile-containing substrates and/or an increased stability of the nitrilase, one or both characteristics increased relative to that of the native microbial gene, the method comprising the steps of

- (i) contacting restriction endonucleases with a mixture of nucleotide sequences to yield a mixture of restriction fragments, the mixture of nucleotide sequences comprising
 - a) a native microbial gene;
 - b) a first population of nucleotide fragments which will hybridize with the nucleotide sequences of the native microbial gene of (i)(a); and

- c) a second population of nucleotide fragments which will not hybridize to the nucleotide sequences of the native microbial gene of (i)(a),
- (ii) denaturing the mixture of restriction fragments of step (i);
- (iii) incubating the denatured mixture of restriction fragments of step (ii) with a polymerase; and
- (iv) repeating steps (i), (ii), and (iii) a sufficient number of times to yield a mutated microbial gene encoding a protein characterized by an increased specific nitrilase activity on nitrile-containing substrates and/or an increased stability of the nitrilase, one or both characteristics increased relative to the nitrilase activity of the native microbial gene.

44. **(Withdrawn)** The method of Claim 43 wherein the native microbial gene is *Acidovorax facilis* 72W and the nitrile-containing substrate is 2-methylglutaronitrile.

45. **(Withdrawn)** A mutated microbial gene encoding a protein characterized by an increased specific nitrilase activity on nitrile-containing substrates and/or an increased stability of the nitrilase, one or both characteristics increased relative to the nitrilase activity of a native microbial gene, the mutated microbial gene produced by the method of Claim 43.

46. **(Original)** The transformed microorganism of Claim 19, wherein the host microorganism is *E. coli* strains MG1655 (ATCC 47076), W3110 (ATCC 27325), MC4100 (ATCC 35695), or W1485 (ATCC 12435).

47. **(Previously presented)** An isolated nucleic acid fragment encoding a polypeptide having the amino acid sequence of SEQ ID NO:5.

48. **(Cancelled)**

49. **(Currently amended)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 90 95 % identity as ~~determined by the Needleman and Wunsch algorithm under default parameters~~ compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5.